Thesis submitted to the

Tamil Nadu Dr.M.G.R.Medical University,

Chennai.

In partial fulfillment towards the award of the degree of

Doctorate of Medicine (DM)

In

Clinical Haematology

For the examinations to be conducted in

August 2014

Department of Clinical Haematology

Christian Medical College, Vellore.

Tamil Nadu, India.

An analysis of efficacy of androgenic steroids in patients with Acquired Aplastic Anemia.

CERTIFICATE

This is to certify that this thesis titled "An analysis of efficacy of androgenic steroids in patients with Acquired Aplastic Anemia," is a bonafide work of the candidate,

Dr. Nisham.P.N, during the period from August 2011 to July 2014 in partial fulfillment, towards the award of degree of Doctorate of Medicine (higher specialty) in Clinical Haematology for the examinations to be conducted by the Dr.M.G.R Medical University in August 2014.

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Sub: **FLUID Research grant project NEW PROPOSAL:**
An analysis of efficacy of androgens (stanozolol&Danazol) in acquired aplastic anemia patients: A single tertiary Centre experience from India.
Dr. Nisham P N, PG Registrar, Clinical hematology, Dr. Biju George, Dr. Alok Srivastava, Dr. Vikram Mathews, Dr. Auro Viswabandya, Dr. Aby Abraham, Dr. Rayaz Ahmed, Dr. Abhijeet Ganapule, Clinical Haematology.

Ref: IRB Min No: 8205 dated 13.02.2013

Dear Dr. Nisham P N,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "An analysis of efficacy of androgens (stanozolol&Danazol) in acquired aplastic anemia patients: A single tertiary Centre experience from India." on February 13, 2013.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Cvs of Drs. Nisham P N, Biju George, Alok Srivastava, Vikram Mathews, Auro Viswabandya, Aby Abraham, Rayaz Ahmed, AbhijeetGanapule.
3. A CD containing documents 1 – 3.

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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.

Yours sincerely

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Secretary (Ethics Committee)
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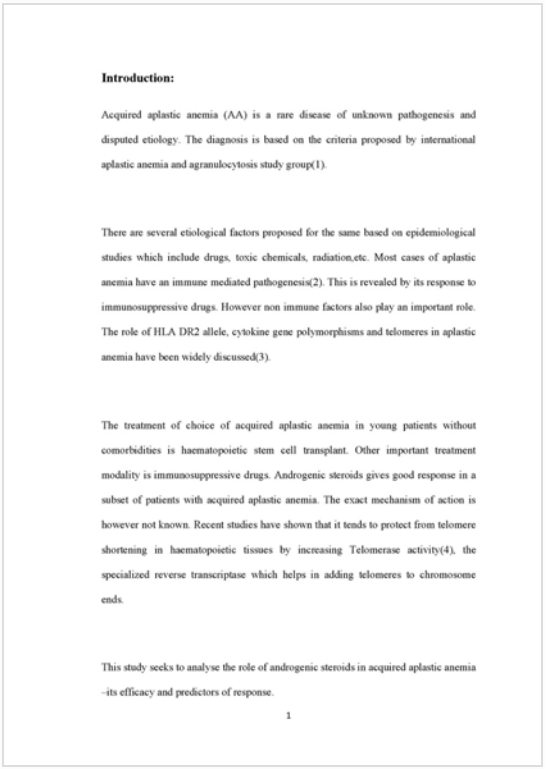

Introduction:

Acquired aplastic anemia (AA) is a rare disease of unknown pathogenesis and disputed etiology. The diagnosis is based on the criteria proposed by international aplastic anemia and agranulocytosis study group(1).

There are several etiological factors proposed for the same based on epidemiological studies which include drugs, toxic chemicals, radiation,etc. Most cases of aplastic anemia have an immune mediated pathogenesis(2). This is revealed by its response to immunosuppressive drugs. However non immune factors also play an important role. The role of HLA DR2 allele, cytokine gene polymorphisms and telomeres in aplastic anemia have been widely discussed(3).

The treatment of choice of acquired aplastic anemia in young patients without comorbidities is haematopoietic stem cell transplant. Other important treatment modality is immunosuppressive drugs. Androgenic steroids gives good response in a subset of patients with acquired aplastic anemia. The exact mechanism of action is however not known. Recent studies have shown that it tends to protect from telomere shortening in haematopoietic tissues by increasing Telomerase activity(4), the specialized reverse transcriptase which helps in adding telomeres to chromosome ends.

This study seeks to analyse the role of androgenic steroids in acquired aplastic anemia –its efficacy and predictors of response.

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(In the name of God, Most Gracious; Most Merciful)

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CONTENTS

Introduction

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The treatment of choice of acquired aplastic anemia in young patients without comorbidities is haematopoietic stem cell transplant. Other important treatment modality is immunosuppressive drugs. Androgenic steroids gives good response in a subset of patients with acquired aplastic anemia. The exact mechanism of action is however not known. Recent studies have shown that it tends to protect from telomere shortening in haematopoietic tissues by increasing Telomerase activity (4), the specialized reverse transcriptase which helps in adding telomeres to chromosome ends.

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Review of Literature

REVIEW OF LITERATURE:

ACQUIRED APLASTIC ANEMIA- DEFINITION AND DIAGNOSIS

Acquired aplastic anemia (AA) is a rare disease of unknown pathogenesis and disputed etiology. In the past there was difficulty in finding out the exact incidence due to lack of strict diagnostic criteria. It is characterized by pancytopenia with other blood cells being morphologically normal. The diagnostic criteria of aplastic anemia and its classification based on its severity is depicted in the table (table: A) below (1).

Table: A. Definition of Aplastic anemia with severity criteria

Classification	Criteria
Severe	BM cellularity < 25% (or <50% if <30% of BM is haematopoietic cells) and ≥ 2 of the following: Peripheral blood neutrophil count <0.5 x 10^9/L Peripheral blood platelet count <20 x 109/L Peripheral blood reticulocyte count <20 x 109/L
Very severe	Peripheral blood neutrophil count <0.2 x 109/L
Non severe	Hypocellular BM , peripheral blood values not meeting the criteria for severe disease.

There may be red cell anisocytosis, toxic granulation in neutrophils and decrease in mean platelet volume all reflecting decreased production of cells in bone marrow (5). In the bone marrow there is increase in fat without increase in reticulin and no increase in abnormal cells. There may be islands of haematopoietic tissue with normal

cellularity along with the hypocellularity. So a bone marrow report showing normal cellularity may sometimes confuse the diagnosis. Aplastic anemia can closely resemble hypoplastic Myelodysplastic syndrome (MDS). Few cases can transform to MDS or acute leukemia and can also develop a clone of PNH (paroxysmal nocturnal haemoglobinuria) cells.

DIFFERENTIAL DIAGNOSIS:

Constitutional marrow failure syndromes like Fanconi anemia and Myelodysplastic syndromes have to be ruled out before the diagnosis of acquired aplastic anemia. Fanconi anemia is diagnosed by morphological abnormalities which will be evident on physical examination and by chromosomal breakage studies. The diagnosis of other rare marrow failure syndromes is more difficult.

MDS is diagnosed by bone marrow morphology and histology and cytogenetic studies. Marked hemophagocytosis, obvious dysplasia, or increased blasts suggests other diseases, although differentiation of hypocellular myelodysplastic syndrome from aplastic anemia can be difficult. Megakaryocytes are the most reliable lineage for distinguishing MDS from SAA. Small mononuclear or aberrant megakaryocytes are typical of MDS, whereas megakaryocytes are markedly reduced or absent in SAA. In contrast, dysplastic erythropoiesis are not uncommon in an aplastic marrow, especially when a PNH is present (6). It is difficult to differentiate aplastic anemia and MDS by bone marrow cytogenetic studies alone because hypocellular marrow of aplastic anemia may not yield sufficient metaphases for cytogenetic studies.

Other important differential diagnosis to be ruled out from history and laboratory tests are aplasia associated with drugs, pregnancy, viruses, PNH, myelofibrosis and

haematological malignancies. Aplastic anemia and PNH overlap in approximately 40% to 50% of cases (the AA/PNH syndrome) (7).

EPIDEMIOLOGY OF APLASTIC ANEMIA:

The first description of a case of Aplastic anemia was by Ehrlich in the later part of nineteenth century, where he described case of a young woman with severe pancytopenia who had a fatal outcome and whose autopsy revealed a yellow marrow. The name aplastic was introduced by Vaquez and Aubertin in 1904 (2). Since then there were various case descriptions of aplastic anemia.

There have been attempts to describe the epidemiology of disease including incidence, mortality and survival trends across the world. There was marked geographic variation in the prevalence of aplastic anemia, most being reported from Asian countries rather than from Europe or United States.

Two large, population-based studies have been conducted, the International Aplastic Anemia and Agranulocytosis Study in Europe and Israel in the 1980s and Thai NHLBI Aplastic Anemia Study in Bangkok and a northeast rural region to find the etiology of aplastic anemia (8). The study was an international collaborative effort with participating centers in Israel, West Germany, Italy, Spain, Hungary, Bulgaria, and Sweden; the total population base was approximately 22 million people. The annual incidence of aplastic anemia ranged from 0.7-4.1 cases per million in these studies. Bangkok had the highest incidence of aplastic anemia when compared to other regions.

The incidence reported by Montané et al. of 2.34/million(9) is similar to the rate of 2.0 for the International Agranulocytosis and Aplastic Anemia Study in Europe and

Israel and Barcelona study is a partial continuation of this. There were similar smaller national studies in France (10), the United Kingdom (11), Scandinavia, (12) and Brazil (13).

However there are no published data on the exact incidence of acquired aplastic anemia among the Indian population.

ETIOLOGY OF APLASTIC ANEMIA:

There were many speculations about the cause of aplastic anemia. Aplastic anemia was previously linked to benzene especially among Swedish bicycle makers (14) , pharmaceutical drug use esp. Choramphenicol, malarial prophylactic drug use (15) etc. From population studies environmental factors appears to be more important than genetic factors in the etiology of Aplastic Anemia.

Many etiologies have been proposed suggesting an immune pathogenesis of aplastic anemia. Pregnancy was found to have a definite correlation and blood counts have been shown to improve after termination of pregnancy (16). Similarly immune activation is thought to underlie aplastic anemia which develop secondary to nonviral hepatitis. There were previous associations of benzene with bone marrow failure. Benzene is known to interfere with immune function (17).

Aplastic anemia occurring as an idiosyncratic reactions to drugs is rare. The genetic differences in drug metabolism can contribute to that. There were reports of carbamazepine and chloramphenicol causing bone marrow failure by the same process. Deletions in the glutathione S Transferase gene involved in carbamazepine metabolism have been implicated (18). No definite mechanism has been found for Chloramphenicol and other drugs like Pencillamine and Gold.

In the International Aplastic Anemia and Agranulocytosis Study, numerous associations between drugs and both dyscrasias were quantified, and the study has proven to be a landmark investigation of these rare diseases. Agranulocytosis has been shown to be primarily a drug-induced disease, with approximately two-thirds of the cases accounted for by associated drugs identified in the study. By contrast, only about 25% of cases of aplastic anemia were accounted for by associated drugs, and the majority remain unexplained. Because of the rarity of the diseases, absolute risks for individual drugs were low.

PATHOPHYSIOLOGY:

Immune factors: Immune hypothesis was suggested decades ago when autologous recovery of marrow function occurs after transplants which fail to engraft pointing towards the conditioning regimen as the treatment for altered immunity. Also observations were that transplants without conditioning regimen failed. This is also obvious from the response of Aplastic anemia patients to immunosuppressive drugs. Exposure to specific environmental precipitants, diverse host genetic risk factors, and individual differences in the characteristics of the immune response likely account for the disease's infrequency, variations in its clinical behavior, and patterns of responsiveness to treatment (2).

Early studies identified that invitro marrow cultures were inhibited by addition of lymphocytes and haematopoiesis improved after removal of these lymphocytes. These effector cells were identified by immunophenotyping as cytotoxic CD8+ T lymphocytes expressing Th1 cytokines (19). These clones have been shown to disappear with successful therapy and reappear with disease relapse. In rare instances a small T cell clone persists after therapy and represents T cell tolerance. The

evidence for T cell mediated attack on bone marrow can be found invivo and invitro. The cytokines like interferon gamma and tumour necrosis factor alpha induce death of CD34+ cells invitro through FAS dependent pathway of apoptosis. Murine models have also been developed, in which infusion of parental lymph node cells to F1 donors induces pancytopenia, marrow aplasia and death. However it is not known how the T cells gets activated in aplastic anemia. Polymorphisms involving cytokine genes (20), mutations of PRF1 (encoding Perforin implicated in familial haemophagocytosis is overexpressed in aplastic marrows) and SAP (implicated in X linked lymphoproliferation, SAP protein levels are diminished in aplastic anemia) have been found in aplastic marrows suggesting a genetic basis for T cell activation.

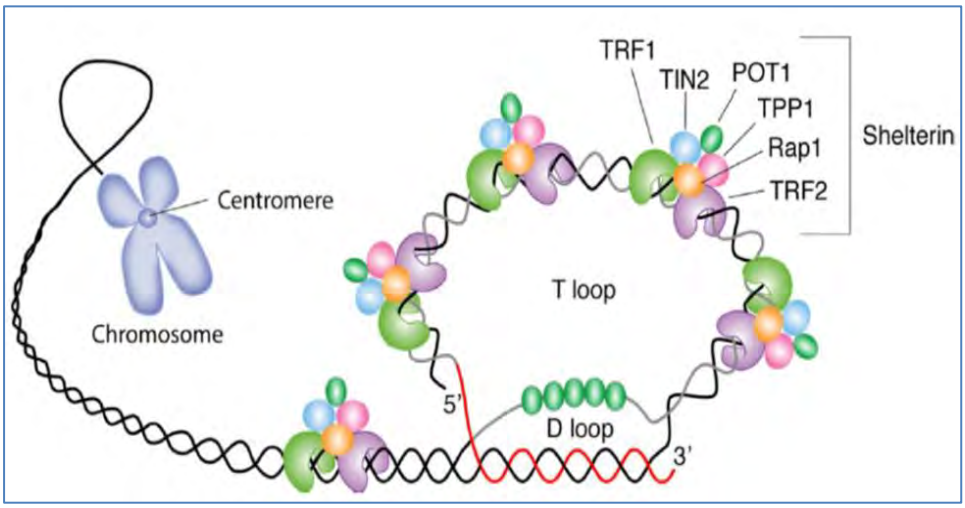
Non Immune Factors: Non immune mechanisms have also been suggested from the failure of people to respond to immune suppressive therapies. Disease refractoriness in such cases has been attributed to depletion of stem cell pool, or immunologic mechanisms not responsive to current treatment. Certain genetic risk factors also appear to contribute to the pathophysiology of Aplastic anemia. Aplastic anemia is linked to HLA class II antigen, DR2 especially in patients who are responsive and dependent on immunosuppressive drugs. Nucleotide polymorphisms in certain cytokine genes like TNF-α, γ-interferon, which is a regulator of immune response, and inherited mutations in the telomerase gene complex has been postulated. The defects in telomere repair leads to progressive telomere shortening and subsequently to marrow failure and aplastic anemia. Telomere shortening was initially blamed to be due to the shrunken stem cell pool in the bone marrow. Mutations in DKC1 and TERC have been identified in X linked and autosomal dominant forms of Dyskeratosis congenital (21), a constitutional marrow failure syndrome. Similar mutations have been found in acquired aplastic anemia. Likewise mutations in SBDS,

gene mutated in Schwachmann Diamond syndrome which has short telomeres have been seen in acquired aplastic anemia. However Telomeres are short in one third to one half of aplastic anemia patients, but such mutations are found in less than 10% patients. This may be due to mutations in other as such unidentified genes involved in telomere repair mechanism or it may be secondary to stem cell replication.

Telomeres and their role in Aplastic Anemia: Structure and function of Telomeres and Related Structures: Telomeres are DNA-protein complexes at the ends of chromosomes that protect the chromosomes from degradation and aberrant recombination of chromosome ends(22). In human beings they are made up of TTAGGG tandem repeats and CCCTAA in the complementary strand. They serve several roles like helping formation of circular ends (t loop), protective protein shield (shelterin) (23), and substitution for coding sequences of important genes at the vulnerable ends of the chromosome. DNA polymerases are unable to replicate the ends of chromosomes, also known as end replication problem. So there is a loss of 50-100kb DNA from the chromosome ends during the process of each cell division. But it can be replicated by polymerases called telomerase, (22) a specialized polymerase with an integral RNA component containing a template element directing the addition of telomeric repeats to chromosome ends.

When telomeres become critically short, protective responses are engaged: short telomeres recruit double-stranded DNA break markers, such as phosphorylated histone H2AX and DNA-damage checkpoint factors, and activate p53 through ATM, up-regulating the cell-cycle inhibitor p21 and blocking cell cycle in G1,.ultimately producing cell proliferation arrest and apoptosis (24). In the absence of telomeres and the cellular protective responses, erosion of the chromosomal ends eventually would cause genomic instability and gene attrition.

Structure of Telomere:



Telomerase Complex: To counter telomere erosion, which was due to an end replication problem, cells with a high proliferative capacity, such as hematopoietic stem cells, express a specialized enzyme, a reverse transcriptase known as telomerase, which helps in extending telomeres by the addition of TTAGGG nucleotide repeats to the 3' terminus of a chromosome's DNA.; the lagging strand is then duplicated by DNA polymerase. The catalytic portion of telomerase contains 2 molecules of each component: the enzyme, telomerase reverse transcriptase or TERT; an RNA template, encoded by TERC; and dyskerin, encoded by DKC1. TERT belongs to a family of reverse transcriptases specialized in elongating telomeres. In humans, the RNA template TERC spans 451 nucleotides, including an 11-nucleotide–long template, and contains several conserved regions essential for its stability. TERC binds to other proteins: dyskerin, GAR, NHP2, and NOP10. Telomeres are capped by at least 6 proteins (TRF1, TRF2, TPP1, POT1, TIN2, and Rap1), collectively known as shelterin, that physically shield the DNA(23). Shelterin allows discrimination of telomeres from double-stranded DNA breaks; lack of shelterin allows telomeres to be

identified as double-stranded DNA breaks and triggers DNA repair. Telomerase is regulated by a wide variety of genes. TERT is repressed by retinoblastoma protein (Rb) and cyclin-dependent kinase inhibitor p21WAF1. Conversely, c-Myc activates TERT gene expression. The TERT promoter contains estrogen receptor elements and in reproductive tissues, estrogen and androgens have important roles in regulating telomerase expression and activity. TERT phosphorylation is another mechanism of telomerase activity regulation. It was recently have discovered that both estrogen and androgens activate telomerase in hematopoietic cells, mediated by estrogen-estrogen receptor complex binding to estrogen response elements in the TERT promoter.

Telomerase is constitutionally expressed in germ line cells and variably expressed in normal haematopoietic stem cells, activated T cells and germinal centre B cells as well as majority of human cancers (25). The length of telomeric repeats has been shown to decrease with cell division both in vivo and in vitro, and there is a age-related decreases in mean telomere length of blood and marrow cells. The reverse of this can also happen, overexpression of telomerase can lead to lengthening of telomeres and immortalization of human epithelial cells and fibroblasts (26).

Studies from UK by Sarah E Ball and colleagues in 1998 tried to analyse whether the marrow failure in aplastic anemia and its transformation to secondary PNH and MDS are the result of the aging process as evidenced by decrease in telomere lengths (27). They found that there is accelerated shortening of telomeres beyond that is expected by age in primary aplastic anemia and secondary MDS patients , but not so much in secondary PNH patients. This was evident in both granulocyte and mononuclear cell populations suggesting that the process would have happened at the level of

haematopoietic stem cell level. There was also stabilization of the telomere shortening after normalisation of blood counts especially following transplants. They suggested further studies in telomere biology for further characteristion of the process. Similar studies were conducted by Neil S Young and Blummendorf in 2001 to measure in Telomere length in leukocyte subpopulations ie. Peripheral blood lymphocytes and granulocytes in patients with aplastic anemia by new method which combines fluorescent insitu hybridization with flow cytometry and found significant telomere length shortening in granulocytes more than that expected by age (28).

Clonal Evolution: Aplastic anemia may coexist or may evolve into PNH or MDS. PNH clone detected in aplastic anemia are small and do not present with the typical hemolysis or thrombosis as classical PNH. Patients who evolve into MDS acquire chromosomal changes like aneuploidy- Trisomy 8, Monosomy 7. Monosomy 7 is the most frequent abnormality and it is associated with poor prognosis. Acute leukaemia has occurred as a terminal event in patients previously shown to have pancytopenia and marrow aplasia (Adams, 1951; Block, Jacobson, and Bethard, 1953). It is uncertain whether these represent two phases of the same illness or whether the fundamental disease is leukaemia with an unusual form of presentation. Paroxysmal nocturnal haemoglobinuria (PNH) is another disease which may occur as a complication of aplastic anaemia, possibly with a greater frequency than hitherto recognized.

MANAGEMENT:

Nonsevere aplastic anemia patients especially those who are not transfusion dependant, need observation alone. Those who progress to severe pancytopenia need treatment with transplantation or immunosuppressive therapy.

Supportive Care: This includes management of infectious and haemorrhagic complications, expectant management of regimen related toxicities, provision of information and psychological support. Important facts like platelet transfusion threshold, (29) spectrum of infections (30) and antibiotic policies have been reviewed and revised frequently. Iron overload doesn't seem to be a significant problem even in transfusion dependent patients. However iron chelation improves the serum ferritin and transaminase levels and in few instances associated with improvement in blood counts (31).

Haematopoietic Stem Cell Transplantation: It is the treatment of choice (32) for young severe and very severe aplastic anemia who has a matched sibling donor. Conditioning regimen included Cyclophosphamide with or without additional agents. The survival of patients undergoing transplant have improved markedly over the recent years (32). Earlier the most important problem faced was graft rejection, which has now decreased with the use of more aggressive conditioning regimens, earlier treatment after diagnosis reducing the number of transfusions and use of less immunogenic blood products. The problem of chronic graft versus host disease (GVHD) still haunts the patient and the physician. In recent reporting by the Center for International Blood and Marrow Transplant Research of more than 1300 SAA patients who were transplanted from 1991 to 2004, survival at 5 years for patients

younger than 20 years of age was 82%, for those 20 to 40 years of age 72%, and for those older than 40 years, closer to 50%.

Immunosuppressive Therapy (IST): Standard initial IST is horse Antithumocyte globulin (ATG) and Cyclosporine-A (CsA), which produces hematologic recovery in 60% to 70% of cases and excellent long-term survival among responders, as shown in several large prospective studies in the United States, Europe, and Japan. A more lymphocytotoxic agent, rabbit ATG, has been successful in salvaging patients with refractory or relapsed severe AA (SAA) after initial horse ATG (33). However, in our recently reported large, randomized controlled study, hematologic response to rabbit ATG (37%) was about half that observed with standard horse ATG (68%), with inferior survival noted in the rabbit ATG arm. ATG is usually administered at a dose of 40 mg/kg over 4 hours, daily for 4 days. Prednisone 1 mg/kg is started on day 1 and continued for 2 weeks, as prophylaxis for serum sickness. Before each ATG dose with acetaminophen and diphenhydramine is given as premedication because infusion reactions are common.

In patients who do not respond to immunosuppressive therapy and who do not have matched sibling donor for a transplant, other options are matched unrelated donor transplant/ (34) umbilical cord blood transplant (34). Another option for patients who have limited resources and no matched sibling will be a trial of androgenic steroids.

Androgens: The introduction of androgens as haematopoietic stimulant for the treatment of aplastic anemia was in 1961 by Shahidi and Diamond (35). Early investigations had already proved the erythropoiesis stimulating activity of Androgens in mammals and Fowls. Also there were observations of increased haemoglobin in

conditions of androgen excess like Cushings syndrome and congenital adrenal hyperplasia and decreased haemoglobin in hypogondism. Similarly the haemoglobin levels were higher in adult males when compared to adult females which cannot be accounted by iron deficiency, pregnancy or blood loss. Another interesting observation was by B J Kennedy and associates who used Testosterone in women with breast cancer and found a high haemoglobin in these patients despite the disease being advanced and metatstatic to the marrow (36). Further androgens were used by Gardner and Pringle in patients with Myeloid Metaplasia (37). It was first used by Shahidi and Diamond in a patient with constitutional aplastic anemia and noted dramatic improvement in blood counts. Further investigations into the mechanism of action of Androgens led to different postulates like stimulation of erythropoietin secretion, stimulation of haematopoietic stem cells for increased DNA synthesis, and increased 2,3-Diphosphoglycerate synthesis which increases oxygen release in tissues. Later there were reports of Shahidi and Diamond regarding benefits of treatment with androgens in patients with acquired aplastic anemia (38). Gardner and Pringle in 1961 described a case of Chloramphenicol induced aplastic anemia who responded to androgens (37).

In a study by Lewis published in 1965 in British Medical Journal from postgraduate medical school in London, there have been instances of remissions in patient receiving testosterone therapy, especially in children near puberty, but this treatment has usually been of less benefit in adult patients, and the ultimate prognosis and median survival seemed little influenced by treatment with moderate doses of androgens and/or steroids (5).

Recently steroids have gone out of use due to lack of effectiveness and increased risk of infection. The androgens in common use recently is Danazol and Stanozolol. There are studies supporting efficacy of both.

Another study from Chandigarh, India in 1991-2000, retrospectively analysed 49 children with aplastic anemia including constitutional aplastic anemia who were treated with Stanozolol and found that none of patients with very severe Aplastic anemia responded to therapy. 28.6% patients with severe aplastic anemia responded and 38% of patients with nonsevere aplastic anemia responded. Median time to respond was 11 weeks. Poor prognostic factors were found to be short history of presentation (<3months) and more than or equal to 70% lymphoid cells in the marrow (39).

In 2011, a study published in Annals of Haematology by Jaime-Perez J C and collegues, studied the effectiveness of Danazol as first line treatment of aplastic anemia. Overall response rate was 46% (17/37) in the danazol-treated group and the median time to initial response was 3 months (1-27). Tendency to achieve remission was similar among severity groups (P = 0.094). The only adverse side effect recorded on the danazol group was an episode of gastrointestinal bleeding (40).

There are also studies showing negative results proving androgens are ineffective. In the study by BM Camitta and collegues in 1979, failed to show any response to androgens. However this study has many limitations, important one being that this study was conducted on patients with only severe aplastic anemia whereas previous studies have shown that androgens work better in patients with nonsevere disease (41).

A study by Freiderick from Boston medical centre of 58 children with acquired aplastic anemia from 1958-70 also speaks against the effectiveness of androgens (42). They treated 57 patients with steroids and a variety of androgenic preparations (sublingual methyltestosterone or testosterone propionate (1-2 mg/kg daily), intramuscular testosterone enanthate (3 mg/kg twice weekly), oral fluoxymesterone (1-2 mg/kg daily), oral oxymetholone (3-6 mg/kg daily), and oral stanozolol (1-2 mg/kg daily)) and supportive therapy. They observed a mortality of 71% over 10 year period. Similarly,in a study by Champlin et al in 1985, compared three treatment groups, patients who received ATG alone, ATG + androgens and ATG historical controls. There was no statistically significant difference in the response rates between three groups if patients are stratified by age, sex, bone marrow cellularity, or etiology of aplastic anemia. Pretreatment peripheral blood counts were also not predictive of response.

An adequate course of androgen is difficult to define, but most clinicians agree that as long as 3 mo of treatment may be required to induce an erythropoietic effect in patients with bone marrow failure of diverse etiologies. A rise in the neutrophil and platelet counts usually may be even more delayed.

The commonly encountered side effects of androgen therapy are virilization, fluid retention, hyperlipidemia, acne, and more seriously, hepatic toxicity with cholestatic jaundice, peliosis hepatitis(42), and rarely, hepatoma (43).

A search for the mechanism of action of androgens and how androgens work in constitutional anemias like Fanconis anemia, Dyskeratosis Congenita and some acquired aplastic anemias have throwed light into its role in increasing Telomerase activity in Haematopoietic tissues. Dyskeratosis congenita, is caused by mutations in

genes involved in telomere maintenance (DKC1 is mutated in X-linked dyskeratosis congenita; TERC, TERT, and TINF2 are mutated in autosomal dominant dyskeratosis congenita; and TERT, NOP10, and NHP2 are mutated in autosomal recessive dyskeratosis congenital (44). Mutations in TERT and TERC have been observed in acquired aplastic anemia (45). They appear to confer a genetic predisposition for the development of aplastic anemia. In vitro studies have been conducted on peripheral blood lymphocytes and bone marrow CD34+ cells in normal and TERT mutated patients. Androgens have been shown to increase telomerase activity as reflected by the increase in TERT mRNA levels in both these group of patients (4). The TERT promoter region contains an imperfect palindromic ERE at position -2677 and an ERE adjacent to an Sp1 site (Sp1-ERE) at position -873. Androgens may act on telomerase expression in hematopoietic tissue after conversion in peripheral tissues to metabolites, such as 17b-estradiol and 5a-dihydrotestosterone; leukocytes express aromatase (CYP19), the enzyme responsible for aromatization of the testosterone (46). Estrogen receptor α , not estrogen receptor β is known to bind to these EREs and promote Telomerase gene expression (47). For the same reason Tamoxifen, the drug used in Breast cancer is known to inhibit both estrogen and androgen effects on Telomerase. But Letrozole, an aromatase inhibitor blocks only androgenic action. Like androgenic compounds , even estrogens may be even more effective in the future for the treatment of bone marrow failure syndromes. This is suggested by reports of bone marrow failure states remitting after the onset of puberty and the onset or relapse of bone marrow failure states during pregnancy when estrogen levels drop.

SUMMARY:

The treatment of acquired aplastic anemia includes mainly allogeneic stem cell transplantation and immunosuppressive therapy (48). Androgenic steroids can be tried in transplant ineligible and resource constraint situations. There are several studies supporting the same. The response to androgenic steroids was more in the nonsevere group of patients. Though the mechanism of action of androgens is still vague, there are several postulations regarding the same. TERT and TERC gene mutations which is commonly seen in patients with Dyskeratosis congenital (21), a constitutional bone marrow failure syndrome, have been found in some patients with acquired aplastic anemia. Androgens have been shown to act on estrogen receptor alpha (androgens gets converted to estrogens via aromatization in the periphery) and activate the TERT gene , thereby increasing the telomerase levels which is known to decrease aging of the haematopoietic tissues and hence apoptosis (4). Further studies are going on in this direction.

Aims and Objectives

Aims and objectives:

1. To analyze the clinical profile of patients (adults and children) with acquired aplastic anemia treated with androgenic steroids.
2. To assess the response to androgenic steroids in the above patients with acquired aplastic anemia.
3. To identify the demographic, clinical, and laboratory parameters that can predict response to androgenic steroids in the above mentioned patients.

Patients and methods

Patients and Methods:

This study protocol was approved by our Institutional Review Board (IRB). This is a retrospective analysis of patients diagnosed to have acquired aplastic anemia and treated with androgenic steroids from January 2008 to December 2012.

Duration of the study: June 2013 to March 2014.

Settings of the study: Department of Clinical Haematology.

Diagnostic criteria: Aplastic Anemia was diagnosed in patients presenting with cytopenia(s) (defined by International Agranulocytosis and Aplastic anemia study group in 1987 as presence of at least two of the following ie. Haemoglobin < 100g/L, neutrophil count < 1.5 $x10^9$/L and platelet count < 50 x 10^9/L) associated with a hypoplastic bone marrow (ie.bone marrow cellularity <25%, <50% if less <30% of the bone marrow is haematopoietic cells) (49).

Patients:

Inclusion Criteria:

1. All adults (age≥15 years) and children (age 1-14 years) diagnosed to have acquired Aplastic anemia and treated with androgenic steroids from January 2008 to December 2012, and with a follow up of at least 12 weeks.

Exclusion Criteria:

1. Patients with inherited bone marrow failure syndromes
2. Patients who had been on any other medications prior to starting androgenic steroids.
3. Patients on androgenic steroids with less than 12 weeks follow up.
4. Patients with acquired Aplastic anemia and on androgenic steroids whose data are not retrievable.

Methods:

Collection of data: After approval by the IRB, the patient data base at our institution was reviewed to identify all patients (adults and children) diagnosed to have acquired Aplastic anemia at our institute from January 2008 to December 2012. Medical information regarding the clinical/laboratory details at diagnosis, post treatment response and adverse events were obtained from the hospital records (laboratory reports/ physician documentation in hospital charts/hospital discharge summaries). Only patients who had at least 12 weeks follow up after initiating therapy were categorized as ‘evaluable‘ for assessment of response and survival.

Treatment: All patients who received androgenic steroids (ie; Danazol nad Stanozolol) as upfront therapy were included in the study. The dosages of the drugs prescribed to the patients were as follows; ie Danazol (400mg to 600mg per day in 2 to 3 divided doses in adults, and 5mg/Kg/day in children), Stanozolol (20 – 60mg per day in 2 to 3 divided doses in adults and 1mg/Kg/day in children). Data was collected with regard to type of treatment, duration of treatment, side effects and overall outcome with respect to the treatment given.

Data analysis: Results are analyzed in terms of the clinical characteristics and laboratory parameters at diagnosis, response to the androgenic steroids, the survival patterns and the parameters predicting response to androgenic steroids. The response to treatment is assessed in terms of Complete Response (CR), Partial response (PR), No response, and death. CR in severe aplastic anemia was defined as haemoglobin normal for age, neutrophil count >1.5 x 10^9/L and platelet count >150 x10^9/L (50). Partial response in severe aplastic anemia is defined as transfusion independence or no longer meeting the criteria for severe disease (50). Complete response in nonsevere aplastic anemia is defined by the same criteria as in severe aplastic anemia(50). Partial response in nonsevere aplastic anemia is defined as transfusion independence or doubling or normalisation of atleast one cell line or increase in baseline haemoglobin > 30g/L (if initially <6), or increase in the baseline neutrophils > 0.5x 10^9/L (if initially <0.5) or increase in the baseline platelet >20 x 10^9/L (if initially <20 (50).

All patients started on treatment with androgenic steroids and with a minimum follow up of 12 weeks were considered evaluable for response and outcome. The closing date for analysis was March 31, 2014.

Statistics: Descriptive statistics were calculated for all variables. Differences in proportions were assessed using the chi-square statistic or Fisher exact test. Differences in means were tested using a t-test or Mann-Whitney-U test as appropriate. The relationships of clinical features to the outcome of the procedure were analyzed by univariate logistic regression model. For all tests, a 2-sided P-value of 0.05 or less was considered statistically significant. SPSS 16.0 software was used for the analysis.

Results

RESULTS:

Between January 2008 and December 2012, a total of 8980 out patients were seen in the Haematology department, of which 1065 (3.67%) were diagnosed to have Aplastic anemia – both constitutional and acquired. Of this only 97 (9.1%) patients received androgenic steroid as first line treatment, while 208 (19.5%) received ATG, 334 (31.4%) were given CSA, 84 (7.9%) underwent Allogeneic PBSCT and the remaining 342 (32.1%) patients had received androgenic steroids after receiving other therapies.

There were a total of 97 patients diagnosed to have acquired aplastic anemia and who received androgenic steroids as initial therapy who fulfilled the inclusion criteria for the study. Out of this 77 (79.3%) were adults (age ≥15 years) and 22 (20.7%) were children (age 1-14 years).

DEMOGRAPHY & CLINICAL FEATURES AT DIAGNOSIS: (Table: 1)

The median age of the 97 patients was 30 years (range: 1-79 years). Twenty (20.6%) patients belonged to the age group of less than 15 years . Males were predominantly represented in the study group i.e. 55 (56.7%) males and 42 (43.3%) females. The male female ratio was 1.3:1. They were patients from different states of the country and two patients from outside country (Bhutan).

Fatigue (due to anemia) and bleeding manifestations were the common presenting symptoms; i.e. in 87.6% (n=85) and 60.8% (n=59) respectively. Bleeding manifestations were mainly minor muco-cutaneous bleeds with 3 (3%) patients

presenting with life threatening bleed ie; one patient presented with sub-arachnoid haemorrhage and 2 patients were with intra-cerebral bleed

Thirty five (36.1%) patients had history of infections. This included mainly recurrent febrile episodes with no obvious focus. None of them had presented with life threatening infections. The median duration of symptoms was 60 days (range: 11-730 days).

All patients except one had received blood product supports (packed red cells and or platelet rich concentrates) prior to presentation to our Institution.

No patient gave history of exposure to drugs, toxic chemicals, prior history of cancer or their treatment either chemotherapy or radiotherapy. Six patients (6.2%) had history of jaundice prior to onset of illness which was treated elsewhere. The median duration of onset of jaundice to symptoms of aplastic anemia was three months. None of these six patients were positive for viral serology.

LABORATORY PARAMETERS AT DIAGNOSIS: (Tables: 2 and 3)

All 97 patients had their diagnosis confirmed by the International Agranulocytosis and Aplastic Anemia Study Group diagnostic criteria described before. Bone marrow examination (aspirate and biopsy) was done in all patients.

Majority of the patients 62 (63.9%) presented with pancytopenia. The median hemoglobin at presentation for the entire cohort was 6.9g/dl (range: 1.7-14.1g/dl), with 37 (38.1%) presenting with a hemoglobin <6g/dl. The median white cell count for the 97 patients was 3300/mm^3 (range:700-13300), while the median absolute neutrophil count (ANC) for these patients was 870 mm^3 (range: 0-9975). The ANC

was <500/ mm^3 in 28 (28.8%) patients while 7 (7.2%) out of this had an ANC <200/ mm^3, falling into the category of very severe aplastic anemia (VSAA). The absolute reticulocyte count (ARC) was available in 79 (81.4%) patients. The median ARC for these patients was 4012 / mm^3 (range: 199-61537), and majority 78 (98.7%) had a count below 20000/ mm^3. The median platelet count at presentation for the entire cohort was 8500/ mm^3 (range: 2000-46000), with 79 (81.4%) presenting with a severe thrombocytopenia (below 20000/ mm^3).

On categorizing the patients according to the severity (table:3), majority (63.9%; n=62) belonged to the severe group, while 28 (28.9%) were non-severe and 7 (7.2%) had very severe Aplastic anemia at diagnosis.

TREATMENT AND RESPONSE: (Tables: 4 and 5; Figure: 1)

Among the total 97 patients included in the study, 83 (85.6%) opted for treatment with androgenic steroids because of financial reasons, while 14 (14.4%) was started on androgens as a bridge therapy before taking up for allogeneic stem cell transplant or ATG therapy, .

Out of the 97 patients, 59 patients (60.8%) received Stanozolol and 38 patients (39.2%) received Danazol. All but 4 of the patients in the Stanozolol group were males (n=55) while all patients in the Danazol group were females (n=38). Response to therapy was assessed at 3months, and 6 months. Adults (≥ 15 years) and children (1-14 years) are analysed as a single group for all the parameters, while the response to treatment has been analysed separately for adults and children. At 6 months follow

up, there were 27 patients who were lost to follow up, and there were excluded from analysis at that time point.

Among adults, at 3 months none of the patients attained complete response, 23 patients (29.9%) showed partial response, 50 patients (64.9%) had no response and data was incomplete in four patients (4.2%). At 6 months, 1 adult patient (3.3%) who was in PR at 3 months showed complete response, while 30 patients (38.9%) were noted to be in PR. Among these patients 10 patients were already in PR at 3 month post treatment, while 20 patients with no response at 3 months had attained PR at 6 months. Fourty six (59.7%) patients showed either no response to treatment (n=26) or were lost to follow to follow up (n=20) at 6 months.

The median time to response was 3 months (range; 1-6 months) in both Stanozolol and Danazol group. The median duration of follow up was 15 months (3-60 months).

Among children at 3 months follow up, none of the patients attained complete response, 7 patients (35%) showed partial response and 13 patients (65%) had no response to treatment. At 6 months, 1 child (5%) had shown complete response (this child was in PR at 3months follow up). Seven (35%) out of the 20 children showed PR at 6months (of these six children were in PR at 3 months while one was in NR at 3 months follow up post treatment)., and 5 patients (60%) had no response to treatment at 6 months.

The median time to response was 3 months (1-6 months) in the Stanozolol group, while in the Danazol group the median time to response was shorter ie; 2.5 months (2-3 months; p value=0.721). The median duration of follow up was 15 months (3-60 months).

Out of the cohort of 97 patients, at six months follow up, 40 patients (41.2%) were responders (CR+PR), and 57 patients (58.8%) were non-responders.

FACTORS PREDICTING RESPONSE TO TREATMENT: (Table: 6).

In the present study, none of the patients who showed response to treatment had lost response while on follow up. All patients who showed response (either PR or CR) were considered as responders even if they were subsequently lost to follow up. All those who did not show response till last follow up date were considered as non-responders (NR). A detailed analysis was done of factors affecting response. No significant association was found with age (p=0.927), gender (p=0.127), duration of illness (p=0.119), ANC (p=0.444), Absolute reticulocyte count (p=0.781), or severity of illness (p=0.266). However, the better response rate noted in the Danazol group (52.6% versus 33.9%) was found to be near significant (p=0.069).

TREATMENT RELATED MORBIDITY: (Table: 7)

Fifteen (15.4%) patients had developed deranged liver function (transaminitis) tests while on follow up. Out of these only two patients (2.1%), had significant (more than 5 times the normal) transaminitis leading to stoppage of the drug; both were in the Stanozolol group. None of the patients had documented hirsutism, voice change, acne or gastric irritation which is other common side effects of androgenic steroids. 19 (19.6%) patients had infections requiring hospitalization during the follow-up period. Out of this 7 patients had bacterial sepsis with documented bacteremia, while 4 were life threatening with septic shock requiring ICU admission. Suspected fungal pneumonia was found in 5 patients (based on radiological evidence), while one

patient had tissue proven rhinocerebral mucormycosis. The rest of the patients had a host of other infections ie; gastro-enteritis (n=3), Gluteal abscess (n=1), pyogenic arthritis (n=1), urinary tract infection (n=1), hydradenitis (n=1) and osteomyelitis (n=1).

Eleven patients (11.3%) had bleeding requiring hospitalization during the follow-up period which included intra-cerebral (n=4), gastrointestinal (n=3) and genitourinary bleeding (n=2) and oro-pharyngeal (n=2).

TREATMENT STATUS AT LAST FOLLOW-UP: (Table 8)

At last follow up 63 (64.9%) patients (53 adults and 10 children) were still on androgens, while 34 (35.1%) patients had either stopped treatment or opted for other treatment modalities or were lost to follow up. Out of the total cohort, 40 (41.2%) were responders (CR+PR) while 57 (58.8%) were non-responders.

23 out of the 57 non-responders had opted for other treatment modalities ie'; allogeneic stem cell transplant [SCT] (n=5), Antithymocyte globulin (n=9), or Cyclosporine (n=9).

All patients who underwent allogeneic SCT had matched related donors; four patients (80%) were children. Four out of the five patients achieved complete response post allogeneic SCT. The child who failed SCT had engraftment failure post second transplant and died subsequently of sepsis.

Out of nine patients (11.1%) who received Antithymocyte globulin, one had complete response, three had partial response and five had no response.

Two (22.2%) of the nine patients who received Cyclosporine had partial response, while the rest had no response.

SURVIVAL:

The median duration of follow up of the entire cohort of patients (n=97) was 15 months (3- 60months). All except two patients were alive at the time of last visit. One patient who died had refractory septic shock due to overwhelming infection and the other patient died of refractory sepsis following engraftment failure post second stem cell transplant. The mortality rate observed in this study do not reflect the actual scenario as there was no follow up data for many patients. An attempt to trace these patients could not be made due to technical reasons (non –availability of proper contact details). None of patient who responded to treatment at any point of time during the study period, had lost response during the subsequent follow up period. None of the patients had progressed to Myelodysplastic syndrome or acute leukemia during the entire follow up period.

TABLES AND FIGURES:

Table: 1 DEMOGRAPHY & CLINICAL FEATURES AT DIAGNOSIS (n = 97)

Variable	n (%) /median (Range)
Median age at diagnosis in years	30 (1-79)
Gender	
Male	55 (56.7)
Female	42 (43.3)
Symptoms at diagnosis	
Fatigue (due to anemia)	85 (87.6)
Bleeding*	59 (60.8)
Infection	35 (36.1)
Duration of symptoms at diagnosis in days	60 (11 -730)

***** Three patients had life threatening bleed. One patient presented with sub-arachnoid haemorrhage and 2 patients were with intra-cerebral bleed.

Table 2: LABORATORY PARAMETERS AT DIAGNOSIS (n=97)

Variable	n (%) /median (Range)
Haemoglobin (g/dl)	6.9 (1.7-14.1)
Total White Cell Count (cells/mm^3)	3300 (700-13300)
Absolute Neutrophil Count (cells/mm^3)	870 (0-9975)
Absolute Reticulocyte Count (cells/mm^3) **(n=79)**	4012 (199 -61537)
Platelet Count (cells/mm^3)	8500 (2000-46000)

Table 3: CLASSIFICATION OF SEVERITY* AT DIAGNOSIS (n=97)

Severity	n (%)
Non Severe	28(28.9%)
Severe	62(63.9%)
Very Severe	7(7.2%)

*International Aplastic Anemia Study Group classification

TREATMENT AND RESPONSE

Table 4: RESPONSE TO ANDROGENIC STEROIDS IN ADULTS (n=77)

	Stanozolol n (%)	Danazol n (%)	**P value**
No. of patients	44 (57.1)	33 (42.8)	-
Male:Female	41 : 3	0 : 33	-
Response status (n=73)*			
At 3 months			
CR	0 (0%)	0 (0%)	-
PR	9 (20.4)	14 (42.4)	**0.020**
NR	35 (79.6)	15 (45.4)	-
At 6 months (n=57)			
CR	1(3.3)	0 (0)	-
PR	12(40)	18(66.7)	0.105
NR	17(56.7)	9(33.3)	-
Time to response in months; median (range)	3(1-6)	3(1-6)	0.678

* In four patients, data was incomplete

Table 5: RESPONSE OF ANDROGENIC STEROIDS IN CHILDREN (n=20)

	Stanozolol n (%)	Danazol n (%)	**P value**
No. of patients	15 (75)	5 (25)	-
Male : Female	14:1	0:5	-
Response status (n=20) **At 3 months**			
CR	0 (0)	0 (0)	1.0
PR	5 (33.3)	2 (40)	-
NR	10 (67.7)	3 (60)	-
At 6 months (n=13)			
CR	1(11.1)	0 (0)	-
PR	5 (55.6)	2 (50)	0.719
NR	3 (33.3)	2 (50)	-
Time to response in months; median (range)	3 (1-6)	2.5 (2-3)	0.721

Table 6: FACTORS PREDICTING RESPONSE TO TREATMENT (n=97)

Variables	Responders (n=40) n (%) / Median(range)	Non-responders (n=57) n (%) / Median (range)	RR	95% CI	P-value
Median age at diagnosis (yrs)	31 (1-79)	30 (2-70)	1.0	0.97-1.02	0.927
Sex: Male	19(34.5)	36(65.5)	1.0		
Female	21(50)	21(50)	1.9	0.83-4.31	0.127
Duration of Illness:					
<60 days	24 (49)	25 (51)	1.0		
>60 days	16 (33.3)	32 (66.7)	0.5	0.23-1.18	0.119
Drug type:					
Stanozolol	20 (33.9)	39 (66.1)	1.0		
Danazol	20 (52.6)	18 (47.4)	2.2	0.94-4.99	**0.069**
Severity of disease:					
SAA& very SAA	26 (37.7)	43 (62.3)	0.6		
Non-severe AA	14 (50)	14 (50)	1.0	0.25-1.46	0.266
Median ANC at diagnosis (cells/mm^3)	889 (0-9975)	816 (120-5390)	1.0	1.00-1.00	0.444
Median ARC at diagnosis (cells/mm^3) [n=79]	4780 (199-12301)	3411 (700-61537)	1.0	1.00-1.00	0.781

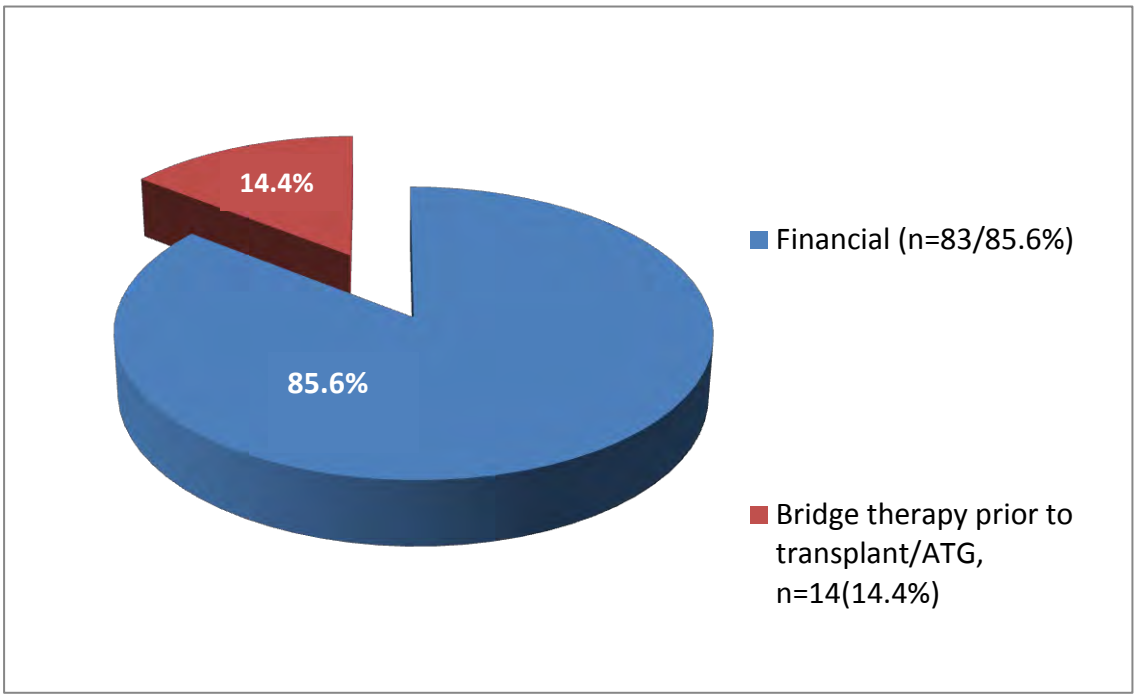
Table 7: MORBIDITY & MORTALITY:

Variables	n (%)
Infections	19 (19.6)
Bleeding manifestations	11 (11.3)
Drug toxicity-Transaminitis	15 (15.5)
Transaminitis with stoppage of therapy	2 (2.0)
Mortality	2 (2.0)

Table: 8 TREATMENT STATUS ON FOLLOW UP (n=97)

	Adults (n=77) n (%)	Children (n=20) n (%)	Status at change of treatment		Response to second line treatment		
			PR n (%)	NR n (%)	CR n (%)	PR n (%)	NR n (%)
Continued on androgens	53 (68.8)	10 (50)	-	-	-	-	-
Changed to ATG	7 (9)	2 (10)	1 (11.1)	8 (88.9)	1 (11.1)	3 (33.3)	5 (55.6)
Changed to CSA	7 (9)	2 (10)	0	9 (100)	0	2 (22.2)	7 (77.8)
Underwent Allog. PBSCT	1 (0)	4 (20)	0	5 (100)	4 (80)	0	1 (20)
Unknown	9 (11.7)	2 (10)	-	-	-	-	-

Figure: 1 REASON FOR CHOICE OF ANDROGENS



Discussion

DISCUSSION

Aplastic anemia is a heterogenous disease with wide variations in outcomes, with some people being transfusion independent and surviving longer while some others having a rapid downhill course culminating in death. In the present scenario there is no doubt regarding the treatment, in that for transplant eligible who have a fully matched sibling, stem cell transplantation is the most efficacious. However in transplant ineligible patients either because of age, comorbidities, lack of a donor or finances, medical therapies including androgens are a viable option. Here an attempt was made to study the demographic profile and response to androgens in treatment naïve patients who were treated in Christian Medical College, Vellore from January 2008 - December 2012.

During the study period there were 28980 patients seen in the outpatient unit of department of Haematology, out of which 1065 (3.67%) cases were diagnosed to have Aplastic Anemia. This study includes 97 treatment naïve patients who opted for androgens as first line treatment.

The median age of the 97 patients studied was 30 years (range: 1-79). Twenty (20.6%) patients belonged to the age group of less than 15 years. Males were predominantly represented in the study group i.e. 55 (56.7%) versus 42 (43.3%) females. In a study published in 1965 in British Medical journal by Lewis et al from Postgraduate Medical School in London, the youngest patient was 2 years old and the oldest was 80. There was no significant preponderance of either sex in the series as a whole or in any age-group, except for a (presumably chance) finding that all the patients between the ages of 35 and 40 were women (5). There was no such preponderance in this study. The data from the international study group of aplastic

anemia and agranulocytosis published in 2008 shows that the median age of patients at diagnosis was 53 years (95% confidence interval [CI] 44–58; range, 2-90). The median age group in this series was reported to be older than that observed in the present study (53 years versus 30 years) (49) . The younger age in this study may reflect the distribution of population as well as reflects the influence of different environmental and genetic factors contributing to the disease.

The median duration of illness from onset to diagnosis in the present study was 60 days. Though neutropenia is common, infection as presenting symptom is very rare (young et al, 2012) (48). This is true in this study where most common symptom is fatigue (87.6%), followed by bleeding (60.8%) and infection (36.1%). But in the case series by Goswami et al (2009) from Northern districts of west Bengal, symptoms of anemia was seen in all cases, fever in 76.1% cases and bleeding in 47.61% cases.

Many drugs and toxic agents have been proposed as etiology in a case of aplastic anemia. No exposure to these drugs or toxic chemicals was evident from the history in this study. In the prospective multicenter study from Barcelona, out of the 235 cases, 49 (20.8%) had history of exposure to drugs and 21 (8.9%) had exposure to toxic agents. The fact that this is a retrospective study and the group studied was small is the limitations to these data.

Six patients (6.2%) had history of jaundice prior to onset of illness which was treated elsewhere. The median duration of onset of jaundice to symptoms of aplastic anemia was three months. None of them were noted to be positive for hepatitis virus serology. Four of these six patients were males and had a median age of 35 years. There was no difference in the outcome of this group of patients compared to the rest. This data is similar to the data published by Safadi et al, in 2001, which showed an interval

between onset of hepatitis and that of AA of 14–225 days (mean 62.3). Length of the interval was unrelated to age, sex or severity of hepatitis. Hepatitis associated Aplastic anemia (HAAA) cases reported in previous studies were generally younger (18 to 20 years) than in the present study (ie; 35 years). Most were male, similar to this study (51).

Majority of the patients 62 (63.9%) presented with pancytopenia. The median hemoglobin at presentation for the entire cohort was 6.9g/dl (range: 1.7-14.1g/dl), with 37 (38.1%) presenting with a hemoglobin <6g/dl. The median white cell count for the 97 patients was 3.300/mm^3 (range:700-13300), while the median absolute neutrophil count (ANC) for these patients was 870 mm^3 (range: 0-9975). The ANC was <500/ mm^3 in 28 (28.8%) patients while 7 (7.2%) out of this had an ANC <200/ mm^3, falling into the category of very severe aplastic anemia (VSAA). The absolute reticulocyte count (ARC) was available in 79 (81.4%) patients. The median ARC for these patients was 4012 / mm^3 (range: 199-61537), and majority 78 (98.7%) had a count below 20000/ mm^3. In the present study, all patients had thrombocytopenia and the median platelet count at presentation for the entire cohort was 8500/ mm^3 (range:2000-46000), with 79 (81.4%) presenting with a severe thrombocytopenia (below 20000/ mm^3). In the study by Gupta and Tripati from BHU, Varanasi in 2008 on 45 patients with AA, the mean hemoglobin was 3.3 g/dL (range 2-6 g/dl) which was much lower than the values in this study (52). The higher hemoglobin value observed in the present study may be because of the prior transfusions these patients had received before presenting to our Institute. However the mean total leukocyte count (TLC) & absolute neutrophil count (ANC) were similar to that observed in the present study ie; 2.8×10^9 /L, 0.6×10^9 /L. In a study by Lewis et al in 1965, the anaemia was usually severe, with lowest levels of haemoglobin between 3 and 8.8

g./dl(5). Almost all cases had an absolute reticulocytopenia at some stage. This is similar to the present patient group in that all patients had reticulocytopenia at diagnosis. The neutrophil count was also usually low, but in half the cases it was normal on occasions. By contrast the platelets were almost invariably lower than normal which is similar to this study.

On categorizing the patients according to the severity groups as proposed by camitta et al in 1976 (41) and Bacigalupo et al in 1988 (53), majority (63.9%; n=62) belonged to the severe group, with 28 (28.9%) belonging to nonsevere and 7 (7.2%) belonging to the very severe group. This distribution is similar to most population studies like the one conducted in Barcelona, Spain in 1980 where 83.8% patients belonged to severe or very severe group.

All ninety seven patients who had atleast one follow up visit at three months after initiation of therapy were evaluated for response. An adequate course of androgen is difficult to define, but most clinicians agree that as long as 3 months of treatment may be required to induce an erythropoietic effect in patients with bone marrow failure of diverse etiologies. A rise in the neutrophil and platelet counts usually may be even more delayed. Fifty nine patients (60.8%) received Stanozolol and 38 patients (39.2%) received Danazol. However a detailed search did not yield any reports comparing Stanozolol with Danazol. The response status for the entire cohort at any point between 3 and 6 months was analysed.

Overall at six months follow up, 40 patients (41.2%) were found to be responders (CR + PR), and 57 patients (58.8%) were nonresponders ie. 37.7% (n=26) patients in severe and very severe AA groups had responded to therapy and 50% (n=14) patients in the nonsevere group had responded to treatment with androgenic steroids. The

median time to response was 3 (1-6) months. Literature review has showed that a study from Chandigarh, India in 1991-2000, retrospectively analysed 49 children with aplastic anemia including constitutional aplastic anemia who were treated with Stanozolol and found that none of patients with very severe Aplastic anemia responded to therapy (39). In our series, 28.6% patients with severe aplastic anemia responded and 38% of patients with nonsevere aplastic anemia responded to treatment. This observation is similar to that in the present study ie; response to androgenic steroid was better in the non-severe groups than the severe or very severe groups. The median time to response reported was also noted to be similar to that in the present study ie 11 weeks versus 12 weeks (in the present study).

Jaime-Perez J C and collegues, studying the effectiveness of Danazol as first line treatment of aplastic anemia in 2011, reported an overall response rate 46% (17/37) in the danazol-treated group with the median time to initial response of 3 months (1-27), reflecting the observation in the present study(40). Tendency to achieve remission was similar among severity groups (P = 0.094). These results match the results of our study where there was no difference between the severity groups (p=0.266). The study by Camitta et al in 1979 totally contradicts our study, in that it failed to show any response to androgens (41). However this study has many limitations, important one being that this study was a retrospective analysis conducted on patients with only severe aplastic anemia whereas previous studies have shown that androgens work better in patients with non-severe disease.

Among adults at 3 months follow up, the responders in the Danazol group was found to be significantly superior to that in the Stanozolol group ie; 42.4% versus 20.4% (p value 0.02). However the response status analysed at 6 months follow up among adults was not observed to statistically significant among the two drug groups (ie;

66.7% in Danazol group versus 43.3% in Stanozolol group; p value= 0.105). Among children no significant difference in response rate was observed between the drug groups at 3 or 6 months. A literature search did not reveal any similar studies for comparison of these observations.

The common side effects of Androgen treatment are virilization, fluid retention, hyperlipidemia, acne, and more seriously, hepatic toxicity with cholestatic jaundice, peliosis hepatitis (42), and rarely, hepatoma (43). In this study the only side effect documented was transaminitis which resolved with stoppage of therapy.

A univariate logistic regression analysis of the common variables was done to detect predictors of response to treatment. This showed no significant association with age (0.927), gender (p=0.127), duration of illness (p=0.119), ANC (p=0.444), Absolute reticulocyte count (p=0.781), or severity of illness (p=0.266) at diagnosis with the response status. However a near significant association was observed with the type of drug (ie; Danazol 52.6% versus Stanozolol 33.9%; p value= 0.069). A similar result can be seen in a study by Champlin et al in 1985, who compared three treatment groups, patients who received ATG alone, ATG + androgens and ATG historical controls (54). There was no statistically significant difference in the response rates between three groups if patients are stratified by age, sex, bone marrow cellularity, or etiology of aplastic anemia. Pretreatment peripheral blood counts were also not predictive of response. Another study by Sanchez Medal in 1966 in two hospitals, consisted of 69 patients who received four different androgens (oxymetholone, methandrolone, metholone and methenolone) (55). In this study, a remission was attained in 33 cases ie. 47.9%. There was no influence of age, sex, bone marrow cellularity or etiology on the response to therapy. A moderately higher rate of remission was observed in females than in males (similar to our study), but the

difference was not significant (p = 0.2). Similarly, a higher remission rate was observed with aplastic anemia secondary to drugs or chemical agents (26 out of 51 cases, or 50.9 per cent) and particularly to phenylbutazone, as compared with those considered as idiopathic. The difference between these remission rates, however, was not significant (p = 0.3 to 0.4). Only the initial reticulocyte per cent value (p=0.001) and the extreme neutropenia showed some relationship with the response to therapy. The degree of anemia or that of thrombocytopenia did not correlate well with response to therapy.

LIMITATIONS OF THE STUDY:

1. Retrospective study: limited available data due to non-retrievable records.

2. This cohort of patients may not be truly representative of general population because of the selection bias, since most of patients presenting to the department opt for definitive therapy-either transplant or immunosuppressive therapy rather than androgenic steroids.

3. Limited patient numbers and data available due to loss of follow up of patients.

Conclusions

CONCLUSION:

1. Androgens still remains a viable option in the treatment of aquired AA in resource constraint situations, with no major drug related adverse effects.
2. There is no significant association between severity and response to androgens.
3. Response to Danazol is superior to Stanozolol in adult patients.
4. Further prospective studies are needed to delineate the mechanisms involved in androgen action and the factors predicting response to therapy.

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Master chart

MASTER CHART:

SlNo	YOD	Age	Sex	Infection	Bleeding	Fatiguability	Duration of illness(days)	Major Bleeding	Serious Infection	Drug Exposure	Chemical Exposure	Jaundice History	Tobacco	Alchohol	Family History	infecton
1	2009	64	F	Y	Y	Y	25	N	N	N	N	N	N	N	N	N
2	2013	61	M	Y	Y	Y	60	N	N	N	N	N	N	N	N	N
3	2011	26	M	N	N	Y	180	N	N	N	N	N	N	N	N	Y
4	2009	14	M	Y	Y	Y	15	N	N	N	N	N	N	N	N	Y
5	2008	14	M	Y	Y	Y	45	N	N	N	N	N	N	N	N	N
6	2008	32	F	N	N	Y	30	N	N	N	N	N	N	N	N	N
7	2008	27	M	N	N	Y	90	N	N	N	N	Y	N	N	N	N
8	2008	22	M	Y	Y	Y	150	N	N	N	N	N	N	N	N	N
9	2008	59	M	N	Y	Y	365	N	N	N	N	N	Y	Y	N	N
10	2008	30	M	N	Y	Y	75	N	N	N	N	N	N	N	N	N
11	2009	12	F	Y	Y	Y	60	N	N	N	N	N	N	N	N	N
12	2009	46	F	N	Y	Y	210	N	N	N	N	N	N	N	N	N
13	2009	2	M	N	Y	N	365	N	N	N	N	N	N	N	N	N
14	2009	34	M	N	Y	Y	90	N	N	N	N	N	N	N	N	N
15	2009	29	M	Y	N	Y	90	N	N	N	N	N	N	N	N	N
16	2009	40	M	N	N	Y	90	N	N	N	N	N	N	N	N	N
17	2010	54	F	N	Y	Y	180	Y	N	N	N	N	N	N	N	N
18	2010	5	F	Y	N	Y	180	N	N	N	N	N	N	N	N	N
19	2010	52	M	N	N	Y	20	N	N	N	N	N	N	N	N	N
20	2010	70	M	N	N	Y	365	N	N	N	N	N	Y	N	N	N
21	2011	52	F	N	N	Y	30	N	N	N	N	N	N	N	N	N
22	2011	33	M	Y	Y	Y	90	N	N	N	N	N	N	N	N	N
23	2012	19	F	N	Y	Y	365	N	N	N	N	N	N	N	N	N
24	2012	35	F	N	Y	Y	30	N	N	N	N	N	N	N	N	Y
25	2012	49	F	N	N	Y	270	N	N	N	N	N	N	N	N	N
26	2012	57	F	Y	Y	Y	30	N	N	N	N	Y	N	N	N	N
27	2012	27	F	Y	N	N	120	N	N	N	N	N	N	N	N	N
28	2012	63	M	N	N	Y	90	N	N	N	N	Y	N	N	N	N
29	2012	53	M	N	N	Y	210	N	N	N	N	N	N	Y	N	N
30	2013	45	F	Y	Y	Y	270	N	N	N	N	N	N	N	N	N
31	2013	29	M	N	Y	Y	180	N	N	N	N	N	N	N	N	N

Sl No	Hb	WBC	ANC	ARC	Platelet	Severity	Stanl/ Dan	Date of starting	Duration of Trt	Resp. 3 mo	Resp. 6 mo	Resp. status	Time to resp. 6mo	Time to Response	Side Effects	Other Treatment	Current Status	Date of last FU
1	5.4	2400	408	NA	10000	S	S	17-04-2009	6	1	0	0	0	NR	N	N	1	09-10-2009
2	4.6	2400	1200	61537	11000	NS	S	10-05-2013	6	1	1	0	0	NA	N	A	1	14-03-2014
3	3.5	2300	322	1822	6000	S	S	19-04-2011	16	1	1	0	0	8	N	A	1	07-01-2014
4	5	3000	120	2751	5000	VS	S	16-10-2009	6	1	1	0	0	NA	1	A	1	14-01-2014
5	4.7	3300	330	2349	4000	S	S	29-12-2008	6	1	1	0	0	NA	N	C	1	23-07-2013
6	4	3000	1140	2702.2	17000	S	D	29-04-2008	12	1	1	0	0	9	N	N	1	19-05-2009
7	2.7	2800	1456	2466	19000	S	S	15-07-2008	15	1	1	0	0	NA	N	N	1	26-06-2010
8	2.7	5500	825	NA	9000	NS	S	19-08-2008	18	1	1	0	0	18	N	N	1	18-02-2010
9	6.2	2700	1242	4653.6	6000	S	S	29-08-2008	9	1	1	0	0	NA	N	N	1	08-05-2009
10	8.5	3500	1295	NA	5000	NS	S	31-10-2008	51	1	1	0	0	NA	N	C	1	19-01-2013
11	6.3	4400	1100	4118.1	13000	S	D	13-01-2009	5	1	1	0	0	NR	N	T	1	07-11-2013
12	5.6	2300	276	NA	10000	S	D	24-02-2009	12	NA	NA	0	0	NA	N	N	1	26-02-2010
13	12.3	4800	720	3854.4	10000	S	S	19-05-2009	9	1	1	0	0	NR	N	N	1	25-03-2010
14	5.6	2500	900	4653.7	5000	S	S	19-06-2009	12	1	1	0	0	NR	N	N	1	18-05-2010
15	5.4	2700	756	3411.2	3000	S	S	28-07-2009	25	1	1	0	0	19	N	N	1	26-08-2011
16	9.6	7700	5390	5699.2	29000	NS	S	18-08-2009	8	1	1	0	0	NA	N	N	1	25-04-2010
17	6.2	2500	950	NA	11000	NS	S	16-02-2010	20	1	1	0	0	NA	N	C	1	21-09-2011
18	6.9	3900	156	3707.4	6000	VS	D	22-06-2010	27	1	1	0	0	9	N	N	1	12-11-2012
19	5.6	4600	1932	NA	10000	NS	S	22-10-2010	9	1	1	0	0	NA	N	N	1	27-06-2011
20	3.9	4600	1932	NA	7000	NS	S	15-11-2010	12	1	1	0	0	NA	N	N	1	30-11-2011
21	4	5100	1122	4259.8	8000	S	D	13-05-2011	6	1	1	0	0	NA	N	C	1	20-12-2013
22	6.3	3600	1440	NA	8000	NS	S	14-10-2011	15	1	1	0	0	NA	N	N	1	09-01-2013
23	7.5	2700	540	3390.2	9000	S	D	20-01-2012	24	1	1	0	0	21	N	N	1	20-12-2013
24	5.4	6700	4154	2836.2	8000	S	D	19-06-2012	21	1	1	0	0	NA	N	N	1	17-03-2014
25	6.9	2700	1080	5103.5	36000	NS	D	13-03-2012	17	NA	NA	0	0	17	N	N	1	27-08-2013
26	8.8	1800	1008	3718	41000	NS	D	10-07-2012	18	1	1	0	0	9	N	N	1	03-01-2014
27	7.4	4600	2346	5638.6	30000	NS	D	29-06-2012	7	1	1	0	0	NA	N	T	1	17-02-2014
28	7.5	2400	576	2926	7000	S	S	04-01-2013	9	1	1	0	0	NA	N	N	1	17-10-2013
29	7.4	4500	1080	3285.6	19000	S	S	22-03-2013	6	1	1	0	0	NA	N	C	1	04-03-2014
30	7.3	2200	308	5977	5000	S	D	08-03-2013	6	1	1	0	0	NA	N	N	1	04-02-2014
31	11.5	3100	1085	1427.4	7000	S	S	14-06-2013	7	1	1	0	0	NA	N	N	1	22-03-2014

SlNo	YOD	Age	Sex	Infection	Bleeding	Fatiguability	Duration of illness(days)	Major Bleeding	Serious Infection	Drug Exposure	Chemical Exposure	Jaundice History	Tobacco	Alchohol	Family History	infecton
32	2011	25	M	N	Y	Y	30	N	N	N	N	N	N	N	N	N
33	2008	75	F	N	N	Y	60	N	N	N	N	N	N	N	N	N
34	2013	47	F	N	Y	Y	7	N	N	N	N	N	NA	NA	NA	N
35	2008	63	M	N	Y	Y	180	N	N	N	N	N	N	N	N	N
36	2008	41	F	N	Y	Y	90	Y	N	N	N	N	N	N	N	Y
37	2008	35	F	N	Y	Y	120	N	N	N	N	N	N	N	N	N
38	2008	30	M	Y	N	N	365	N	Y	N	N	N	N	N	N	Y
39	2008	8	F	N	Y	N	540	N	N	N	N	N	N	N	N	Y
40	2008	11	M	Y	Y	Y	45	N	N	N	N	N	N	N	N	N
41	2008	22	M	N	Y	Y	120	N	N	N	N	N	N	N	N	N
42	2009	19	M	N	Y	Y	120	N	N	N	N	N	N	N	N	N
43	2009	33	F	N	Y	Y	180	N	N	N	N	N	N	N	N	N
44	2009	33	F	N	N	Y	330	N	N	N	N	N	N	N	N	N
45	2009	56	F	N	N	Y	180	N	N	N	N	N	N	N	N	N
46	2009	79	F	N	Y	Y	210	N	N	N	N	N	N	N	N	N
47	2009	26	F	N	N	Y	30	N	N	N	N	N	N	N	N	N
48	2010	27	F	Y	Y	Y	60	Y	N	N	N	N	N	N	N	N
49	2010	49	M	Y	Y	Y	45	N	N	N	N	N	N	N	N	N
50	2010	45	F	N	N	Y	60	N	N	N	N	N	N	N	N	N
51	2010	50	M	N	Y	Y	30	N	N	N	N	N	N	N	N	N
52	2010	40	F	N	N	N	30	N	N	N	N	N	N	N	N	N
53	2010	56	F	N	N	N	30	N	N	N	N	N	N	N	N	N
54	2010	10	F	Y	N	N	60	N	N	N	N	N	N	N	N	N
55	2011	1	F	Y	N	N	365	N	N	N	N	N	N	N	N	N
56	2011	35	M	N	Y	Y	30	N	N	N	N	N	N	N	N	N
57	2011	32	F	N	Y	Y	30	N	N	N	N	N	N	N	N	N
58	2012	8	M	N	N	Y	60	N	N	N	N	N	N	N	N	N
59	2012	33	M	N	Y	Y	30	N	N	N	N	N	N	N	N	N
60	2012	45	F	N	Y	Y	210	N	N	N	N	N	N	N	N	N
61	2012	16	M	N	Y	Y	720	N	N	N	N	N	N	N	N	N
62	2012	8	M	Y	Y	N	15	N	N	N	N	N	N	N	N	N
63	2012	48	M	Y	N	Y	45	N	N	N	N	N	N	N	N	N
64	2012	24	F	Y	N	Y	15	N	N	N	N	N	N	N	N	N
65	2012	27	F	Y	Y	Y	20	N	N	N	N	N	N	N	N	N

Sl No	Hb	WBC	ANC	ARC	Platelet	Severity	Stanl/ Dan	Date of starting	Duration of Trt	Resp. 3 mo	Resp. 6 mo	Resp. status	Time to resp. 6mo	Time to Response	Side Effects	Other Treatment	Current Status	Date of last FU
32	8.2	4900	588	1558	15000	S	S	12-07-2011	24	1	3	1	4	4	N	N	1	04-11-2013
33	4.9	2800	532	4180	12000	S	D	23-09-2008	29	3	3	1	5	5	N	N	1	13-08-2013
34	8.2	2500	800	4563	6000	S	D	31-05-2013	7	3	3	1	4	4	N	N	1	31-01-2014
35	7	3600	1116	4788	18000	S	S	04-04-2008	6	3	3	1	3	3	N	N	1	28-10-2008
36	11.4	4400	2288	12301	8000	S	D	15-01-2008	6	3	3	1	2	2	N	N	1	17-09-2010
37	7.9	2900	522	9623.4	17000	S	D	02-05-2008	9	3	3	1	3	3	N	N	1	03-02-2009
38	10.8	4600	2254	10392.9	33000	S	S	22-07-2008	60	3	3	1	3	3	N	N	1	06-08-2013
39	10.4	9400	940	NA	8000	NS	S	10-10-2008	9	3	3	1	3	3	N	T	1	28-05-2011
40	7.2	5300	689	4510	11000	S	S	02-12-2008	24	3	3	1	1	1	N	N	1	21-01-2011
41	7	2900	957	4012.8	11000	NS	S	06-01-2009	11	3	3	1	3	NR	N	N	1	07-12-2009
42	10	6600	1386	9519.2	12000	S	S	13-01-2009	48	3	3	1	3	9	N	N	1	21-02-2013
43	6.7	6200	2294	6570.4	20000	S	D	27-01-2009	5	NA	3	1	5	5	N	N	1	02-06-2009
44	11.6	2600	1508	6867.2	46000	NS	D	15-09-2009	6	NA	3	1	6	NR	N	N	1	14-10-2011
45	2.3	4500	2205	NA	11000	NS	D	18-08-2009	36	3	3	1	3	3	N	N	1	26-02-2014
46	12.1	4200	924	NA	8000	NS	D	20-10-2009	39	1	3	1	6	6	N	N	1	11-01-2013
47	9.7	2900	696	2626.5	10000	S	D	01-12-2009	31	3	3	1	2	2	N	N	1	13-07-2012
48	9.9	2100	420	6578	16000	S	D	30-03-2010	16	3	3	1	1	1	N	N	1	20-07-2011
49	4.8	3300	363	NA	5000	S	S	08-06-2010	9	3	3	1	3	3	N	N	1	11-03-2011
50	6.5	1600	896	5582.2	30000	NS	D	31-08-2010	33	3	NA	1	3	9	N	N	1	29-05-2013
51	5.9	2500	275	1417.5	7000	S	S	12-10-2010	14	1	3	1	4	4	N	N	1	13-12-2011
52	10.1	4000	1760	7950	43000	NS	D	03-11-2010	27	3	3	1	3	3	N	A	1	27-01-2014
53	14.1	13300	9975	4474.3	20000	NS	D	27-11-2010	38	1	3	1	6	6	N	N	1	31-01-2014
54	8.1	3100	651	8668.8	9000	S	D	04-12-2010	18	3	3	1	3	3	N	N	1	27-06-2012
55	8.8	5800	870	9828	22000	NS	D	08-02-2011	31	3	3	1	2	2	N	N	1	03-09-2013
56	7.2	2100	882	3821.8	16000	S	S	11-10-2011	25	1	3	1	6	6	N	N	1	09-10-2013
57	5.3	4100	1271	2141.3	8000	S	D	19-04-2013	10	3	3	1	1	1	N	N	1	28-02-2014
58	13.2	2800	1736	6401.4	16000	S	S	23-03-2012	18	3	3	1	3	3	N	N	1	27-09-2013
59	7	4400	968	4772.2	11000	S	S	13-03-2012	20	1	3	1	6	6	N	N	1	02-11-2013
60	7.3	3000	1260	4237	20000	NS	D	21-03-2012	8	3	3	1	2	2	N	N	1	13-11-2012
61	9.1	3800	836	4767.2	23000	NS	S	11-04-2012	18	1	3	1	4	4	N	N	1	25-03-2014
62	5.6	2900	203	4867.8	9000	S	S	29-03-2012	23	3	3	1	2	2	N	N	1	07-02-2014
63	11.2	2200	682	4931.9	18000	S	S	25-09-2012	13	3	3	1	1	1	N	N	1	15-10-2013
64	1.7	3500	350	2160	5000	S	D	02-10-2012	12	3	3	1	3	3	N	N	1	18-10-2013
65	10.5	1600	64	2548.9	29000	VS	D	28-09-2012	15	3	3	1	1	1	N	N	1	31-12-2013

SlNo	YOD	Age	Sex	Infection	Bleeding	Fatiguability	Duration of illness(days)	Major Bleeding	Serious Infection	Drug Exposure	Chemical Exposure	Jaundice History	Tobacco	Alchohol	Family History	infecton
66	2012	22	F	N	N	Y	180	N	N	N	N	N	N	N	N	N
67	2012	13	M	N	Y	Y	11	Y	N	N	N	N	N	N	N	Y
68	2013	24	M	Y	Y	Y	90	N	N	N	N	N	N	Y	N	N
69	2009	13	M	Y	N	N	30	N	N	N	N	N	N	N	N	N
70	2012	41	M	N	Y	Y	60	N	N	N	N	N	N	Y	N	N
71	2013	11	M	Y	Y	Y	30	Y	N	N	N	N	N	N	N	Y
72	2013	28	M	Y	N	Y	10	N	N	N	N	N	NA	NA	NA	N
73	2008	22	M	Y	Y	Y	150	N	N	N	N	Y	N	N	N	Y
74	2008	42	M	N	N	Y	60	N	N	N	N	N	N	N	N	N
75	2008	43	M	N	Y	Y	730	N	N	N	N	Y	N	N	N	N
76	2008	21	M	Y	Y	Y	365	N	N	N	N	Y	N	N	N	N
77	2009	59	F	N	Y	Y	30	N	N	N	N	N	N	N	N	N
78	2009	54	F	Y	N	Y	90	N	N	N	N	N	N	N	N	N
79	2009	8	M	N	Y	Y	15	Y	N	N	N	N	N	N	N	Y
80	2009	25	F	Y	Y	Y	20	Y	N	N	N	N	N	N	N	N
81	2009	11	M	Y	Y	Y	180	N	N	N	N	N	N	N	N	N
82	2009	12	F	N	N	Y	7	N	N	N	N	N	N	N	N	N
83	2009	18	M	N	Y	Y	60	Y	N	N	N	N	N	N	N	N
84	2010	30	F	N	Y	Y	180	N	N	N	N	N	N	N	N	N
85	2010	47	M	N	Y	Y	90	N	N	N	N	N	N	N	N	N
86	2010	34	M	N	Y	Y	30	N	N	N	N	N	N	N	N	N
87	2010	45	F	N	N	Y	120	N	N	N	N	N	N	N	N	N
88	2010	10	M	N	N	Y	21	Y	Y	N	N	N	N	N	N	Y
89	2011	44	M	N	N	Y	210	N	N	N	N	N	N	N	N	N
90	2011	10	M	Y	Y	Y	90	N	N	N	N	N	N	N	N	N
91	2011	8	M	Y	Y	N	180	N	N	N	N	N	N	N	N	N
92	2011	18	M	Y	N	Y	60	N	N	N	N	N	N	N	N	N
93	2012	45	M	N	Y	Y	15	N	N	N	N	N	N	N	N	N
94	2012	23	F	N	N	N	30	N	N	N	N	N	N	N	N	N
95	2012	22	M	N	Y	Y	45	Y	Y	N	N	N	N	N	N	Y
96	2012	26	M	N	N	Y	45	N	N	N	N	N	N	N	N	N
97	2012	21	F	Y	Y	Y	30	Y	Y	N	N	N	N	N	N	Y

Sl No	Hb	WBC	ANC	ARC	Platelet	Severity	Stanl/ Dan	Date of starting	Duration of Trt	Resp. 3 mo	Resp. 6 mo	Resp. status	Time to resp. 6mo	Time to Response	Side Effects	Other Treatment	Current Status	Date of last FU
66	7.5	3900	1365	6487.2	31000	NS	D	27-11-2012	10	3	3	1	3	3	N	N	1	16-08-2013
67	6.8	1400	0	198.9	3000	VS	S	22-01-2013	11	1	3	1	6	NA	N	N	1	15-11-2013
68	5.9	3500	280	1812.8	12000	S	S	26-03-2013	9	3	3	1	3	3	N	N	1	25-02-2014
69	10.4	4700	1175	9668.5	23000	NS	S	16-10-2009	12	3	4	1	3	3	N	N	1	12-06-2012
70	5.6	3300	1221	4107.6	13000	S	S	22-01-2013	11	3	4	1	1	1	N	N	1	07-03-2014
71	.	3000	180	11792	6000	S	S	15-05-2013	3	1	NA	LFU		NA	N	N	1	25-10-2013
72	2.3	700	350	2224	6000	S	S	28-06-2013	3	1	NA	LFU		NA	N	N	1	06-09-2013
73	4.9	2200	176	3628	7000	VS	S	25-01-2008	3	1	NA	LFU		NA	N	C	1	05-09-2008
74	9.6	3900	1521	NA	13000	NS	S	10-10-2008	3	1	NA	LFU		NA	N	C	1	22-01-2009
75	8	3100	1023	3570	10000	S	S	10-10-2008	6	1	NA	LFU		NA	N	N	1	21-03-2009
76	7.2	2000	280	1127	9000	S	S	08-11-2008	3	1	NA	LFU		NA	N	N	1	27-04-2009
77	4.6	2800	280	NA	11000	S	D	17-02-2009	3	1	NA	LFU		NA	N	N	1	26-05-2009
78	7.9	3000	450	3847.9	8000	S	D	24-02-2009	4	1	NA	LFU		NA	N	N	1	08-06-2009
79	10.8	4100	164	700	16000	VS	S	03-04-2009	3	1	NA	LFU		NR	N	C	1	08-01-2010
80	6.3	2400	816	NA	2000	NS	S	07-04-2009	4	1	NA	LFU		NA	N	N	1	27-02-2010
81	9.8	4400	440	910.6	5000	S	S	04-08-2009	3	1	NA	LFU		NR	N	N	1	20-10-2009
82	3.1	3900	1053	1780	9000	S	D	13-11-2009	5	1	NA	LFU		NA	N	T	1	23-01-2014
83	3	3900	507	3390.8	3000	S	S	22-12-2009	4	1	NA	LFU		NA	N	N	0	20-04-2010
84	8	2500	450	6576.6	9000	S	D	13-07-2010	4	1	NA	LFU		NA	N	C	1	27-11-2010
85	5.1	2000	560	2783.2	6000	S	S	16-03-2010	4	1	NA	LFU		NA	N	N	1	23-07-2010
86	4	1900	456	2101.2	5000	S	S	25-06-2010	3	1	NA	LFU		NA	N	N	1	14-09-2010
87	4.5	3100	372	3872.6	9000	S	D	24-09-2010	3	1	NA	LFU		NA	N	N	1	31-12-2010
88	10.6	6200	186	2550	4000	VS	S	26-02-2011	3	1	NA	LFU		NA	N	T	1	07-03-2014
89	6.8	6300	1449	3916.8	9000	S	S	08-04-2011	5	1	NA	LFU		NA	N	A	1	07-03-2014
90	3	4000	800	2668	7000	S	S	01-06-2011	3	1	NA	LFU		NA	N	A	1	30-09-2013
91	5.6	3600	252	NA	12000	S	S	30-09-2011	4	1	NA	LFU		NA	N	N	1	24-01-2012
92	6.2	4400	1056	5837.4	14000	S	S	29-11-2011	3	1	NA	LFU		NR	N	N	1	17-09-2013
93	4.1	1800	288	NA	2000	S	S	14-09-2012	3	1	NA	LFU		NA	N	N	1	20-11-2012
94	10.4	6700	2010	NA	13000	NS	D	27-07-2012	3	1	NA	LFU		NA	N	A	1	15-02-2014
95	7.5	5500	1925	2242	6000	S	S	28-09-2012	3	1	NA	LFU		NA	1	A	1	28-02-2014
96	5.5	2500	650	6146	23000	NS	S	26-04-2008	91	3	NA	LFU		3	N	N	1	13-11-2012
97	7.2	3800	2622	6466	21000	S	D	05-02-2013	4	1	NA	LFU		NA	N	A	1	18-03-2014
	CODING																	
	1= No Response; 3= Partial response; 4=Complete Response. LFU= Lost to follow up																	